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Research Article

Long Term Survival of Chronic Myeloid Leukemia Patients with Chromosomal Aberrations in Philadelphia Negative Metaphases During Treatment with Tyrosine Kinase Inhibitors

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Abstract

Additional clonal aberrations (ACAs) in Philadelphia negative metaphases of Chronic Myeloid Leukemia (CML) patients treated with tyrosine kinase inhibitors (TKIs) were an early noticed event in the course of the treatment plan. However, their biological and clinical implications are not conclusive. In the present study, we investigated their incidence and impact on the prognosis and treatment response. Among 200 CML patients treated in our Department from 2002 to 2019, ACAs in Ph- cells were found in 21 (10.5%), with a median time from initiation of TKIs at 58 months. In addition to monosomy 7, trisomy 8 and loss of chromosome Y, we also identified other numerical and structural abnormalities including add(6)(q27), t(4;15)(q31;q26) t(2;10)(q23;q24), which have never been reported before. We noticed that the number of ACAs developed in Ph- cells is associated to the number of treatments before the initiation of TKIs. Probability of long-term overall survival at 15 years was 87.1%. Persistent clones were revealed in eight patients. As a result, our study indicates that Ph- ACAs do not impact the outcomes of CML. Aberrations such as monosomy 7 need close monitoring due to their association with increased risk of evolution to myelodysplastic syndrome or acute leukemia.

Keywords: Ph(-) clone, Chronic myeloid leukemia; Tyrosine kinase inhibitors

Introduction

Chronic Myeloid Leukemia (CML) is a hematopoietic disorder of multipotential stem cells, hallmarked by the cytogenetic event t(9;22)(q34;q11). It results in the generation of the Philadelphia (Ph) chromosome carrying the BCR-ABL1 fusion gene, which plays a central role in the pathogenesis of CML [1,2]. The initiation of tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL oncoprotein as a first line treatment, has significantly improved the prognosis of this disease. As a result, the survival of CML patients is almost identical to that of the general population [3]. Early during the course of treatment with TKIs, it was found that some patients developed additional clonal aberrations (ACAs) in the Ph+ and Ph- cells [4,5]. Although the development of these ACAs in Ph+ is considered a sign of clonal evolution, suggesting disease progression [4], the clinical and biological significance of their emergence in Ph- cells remains unclear [6,7]. The incidence of ACAs in Ph- cells of various treatment groups is estimated to be 2-17% [8,9]. The most common reported ACAs include monosomy 7, trisomy 8 and loss of chromosome Y [10]. In the present study, we report 21 patients who developed ACAs in Ph- cells. In addition to the common reported aberrations, we also identify other structural and numerical abnormalities, some of which are described for the first time. The results from this investigation may be able to provide further information in the effort to understand the pathogenic and prognostic implications of the clonal chromosomal aberrations in CML.

Materials and Methods

Patients

We conducted a retrospective analysis of patients diagnosed with CML in our Department (Hematology & HCT Unit, General Hospital "George Papanikolaou" Thessaloniki, Greece) from 2002 to 2019. Imatinib, Dasatinib and Nilotinib were the TKIs used in the treatment plan according to our Department's protocols. We systematically recorded data of patients identified carrying ACAs in Ph- cells at some point of their course of treatment with TKIs. The study was conducted according to the Declaration of Helsinki and patients provided written informed consent.

Cytogenetic analysis

Conventional cytogenetic analysis was performed on G-banded metaphase cells prepared from unstimulated 24hr and 48hr bone marrow aspirates cultured using standard techniques. At least 20 metaphases were evaluated for each case when satisfactory cell cultures were available. A clonal cytogenetic abnormality was defined as the same numerical gain or structural abnormalities in at least 2 metaphases or the same numerical loss in at least 3 metaphases. The karyotype was documented according to the International System for Human Cytogenomic Nomenclature (ISCN 2016) [11].

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PCR for BCR-ABL1

BCR-ABL t(9;22) quantitative assay was performed in the Molecular Biology Laboratory in our Department. Briefly, patient RNA was isolated and reverse transcribed to complementary DNA (cDNA). The BCR/ABL and ABL reference gene sequences were amplified in duplicate using multiplexed quantitative real-time PCR. This assay can detect the major, minor, and micro BCR/ABL break-points and has an analytical sensitivity of better than 0.002%. Next Generation Sequencing (NGS) was not performed, due to the fact that most patients were diagnosed before it was available.

Statistical analysis

Data were analyzed using the statistical program SPSS 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Descriptive statistics were performed using median (range) for continuous variables, and frequency for categorical variables. Continuous variables were compared using t-test or Mann-Whitney, according to normality. Categorical variables were compared using the chi-square test. Correlation between continuous variables was assessed with the Pearson's or Spearman's correlation coefficient. Binary or linear logistic regression was used when appropriate. Kaplan-Meier estimates were used to calculate the probability of overall survival (OS). The level of statistical significance was defined at 0.05.

Results

Chromosomal abnormalities

Twenty one out of 200 CML patients (10.5%) were identified carrying ACAs in Ph- cells at some point of their course of treatment with TKIs. The patients' characteristics are summarized in Table 1. Median time from diagnosis to their last follow up was 156 months (range 20-240), and a median time from the beginning of treatment with TKIs, 58months (range 3-204). These ACAs were identified in patients receiving the following TKIs: Imatinib, Dasatinib and/or Nilotinib. Sixteen out of 21 patients were male, suggestive of male predominance, while median age at diagnosis was 49 years (range 20-77). Sixteen patients were diagnosed in chronic phase (CP), 3 in accelerating phase (AP) and 2 in blast crisis (BC).

Among these 21 patients, 15 had a single abnormality. Three patients had 2, two patients had 3, while one patient had 5 chromosomal abnormalities (in 2 patients the abnormalities were found in 2 different clones and in 1 patient in 3 clones). Eighteen patients had only numerical abnormalities, 1 only structural; whereas 2 patients developed both numerical and structural aberrations. We found abnormalities of chromosome Y in 9 patients and more specifically, in 1 as an extra chromosome Y and in 8 as a loss of chromosome Y (as a single abnormality in7). Trisomy 8 was revealed in 9 patients and monosomy 7 in 2 patients. We also found other abnormalities such as +X,+9, +13, +21, add(6)(q27),del(20)(q11), t (4;15)(q31;q26) and t(2;10)(q23;q24). Figure 1A demonstrates a karyotype with ACA t(2;10)(q23;q24).

Examination of the bone marrow aspiration showed normal bone marrow in 7 patients, increase of lymphocytes and/or plasma cells in 6 patients, mild dysmegakaryopoiesis in 3 patients and dry tap in 3 patients. Moreover, none of the bone marrow aspirations and biopsies revealed signs suggestive of myelodysplastic syndrome.

At the end of the study, 2 out of 21 patients died, due to complications irrelevant to CML or TKI treatment, while 3 underwent allogeneic hematopoietic cell transplantation (HCT) - 1 in AP, 1 in BC and 1 in CP but with persistent monosomy 7 in all metaphases. Among the remaining 16 patients still on TKIs, all achieved complete cytogenetic response (CCyR), while 2 are in major molecular response (MMR) and 14 in deep molecular response ($MR^{4.5}$). Furthermore, we investigated the persistence of these ACAs in Ph- cells in these 16 patients. Persistent ACA was defined as continuously positive ACA results in sequential three or more times of cytogenetic follow-up. Eight patients had a persistent clone for a median time of 76 months (range 2-192). Five were found to have loss of chromosome Y, 2 trisomy 8 and 1 patient del(20q),+8 and +9.

Potential association between number of ACAs and number of treatments before TKI initiation

Ten patients with additional chromosomal abnormalities received only TKIs before the emergence of Ph (-) clone, while 11 patients received 1-5 (lines of treatment) prior to the initiation of TKIs. Moreover, the number of these aberrations was associated with the number of treatments before TKIs (p=0.030). Among these patients, 3 had undergone autologous HCT before the initiation of TKIs. Importantly, 2 of them still demonstrate persistent ACAs

Potential association between Overall Survival (OS) and type of BCR-ABL transcript or loss of chromosome Y

Our study revealed that the probability of OS at 15 years was 87.1%. Fourteen out of 21 patients (66.6%) were diagnosed with b2a3 transcripts (e14a2), 6 patients with b2a2 (e13a2) and 1 with b3a2 (e13a3). OS was found to be similar between b3a2 and b2a2 transcripts (p=0.355) as it can be seen in Figure 1B. Additionally, the loss of chromosome Y did not seem to affect OS compared to the non -Y ACAs (p=0.777). Survival measures were not significantly different in patient in which -Y was the only clonal abnormality versus others (p=0.458, 75% versus 92%). Moreover, response to TKI did not affect this difference because all patients in which -Y was the only clonal abnormality achieved optimal response.

Discussion

In this study, we investigated the occurrence of ACAs in Ph- cells in patients with CML. Twenty-one out of 200 patients with history of CML developed chromosomal abnormalities in the Ph- cells during the treatment with TKIs. The observed frequency is 10.5% which is similar to that reported in previous studies [12]. Consistent with previous reports, our study demonstrates that trisomy 8 and monosomy 7/7q- are common chromosomal abnormalities in Ph- cells [10]. Moreover, loss of chromosome Y was also common, appearing in 8 patients (in 7 patients as a single abnormality). In addition to these ACAs, we have detected other rare abnormalities, such as +X, +9, +13, +21, add(6)(q27), del(20)(q11), t (4;15)(q31;q26) and t(2;10)(q23;q24). Importantly, the latter two have never been reported by other studies before according to the International Library (https://mitelmandatabase.isb-cgc.org/).

Most reports demonstrated that the presence of ACAs in the Ph-cells is transient. The persistence of these ACAs was documented in less than 30% of patients [8]. Our study also showed that 8 out of 21 patients (38%) carried persistent chromosomal aberrations as

Table 1: Characteristics of CML Patients with ACAs in Ph- Cells.

Patients	Age	Disease Phase at Dx	Lines of Treatment pre TKIs	Ph-clone	Pre-ACAs duration of TKI treatment (Months)	Persistent ACA (Months)	Current TKIs Treatment	MR Status	Follow-Up Duration (Months)	Current Status/Allo HCT
01. Male	45	CP	2	add(6)(q27)	180	No	Yes	MR4.5	240	ALIVE/NO
02. Male	20	CP	0	-Y	41	No	No	MR4	72	ALIVE/NO
03. Male	40	CP	3	-Y, +13	82	Yes	Yes	MR4	276	ALIVE/NO
04. Male	42	CP	0	-Y, -7	14	No	N/A	MR4.5	162	ALIVE/YES
05. Male	55	BP	3	+X, +8, +19, add(1)(p36),der(6)t(1:6)	10	No	N/A	MR4.5	72	ALIVE/YES
06. Female	47	CP	2	+X, -7, +8, +9, del20	176	Yes	No	MR4.5	108	ALIVE/NO
07. Male	54	CP	0	-Y	132	No	Yes	MR4.5	168	ALIVE/NO
08. Female	77	AP	1	+X, +8	43	No	N/A	N/A	96	DEAD/NO
09. Male	29	CP	3	8	12	No	Yes	MMR	180	ALIVE/NO
10. Male	22	AP	3	-7,	6	No	Yes	MR4.5	66	ALIVE/YES
11. Female	48	BP	3	8	108	No	Yes	MR4.5	216	ALIVE/NO
12. Male	45	CP	2	-Y, t(4;15)(q31:q26), t(10;12)	204	Yes	Yes	MR4.5	216	ALIVE/NO
13. Female	65	CP	0	8	120	No	No	MR4.5	180	ALIVE/NO
14. Male	49	CP	0	-Y	44	Yes	Yes	MR4.5	180	ALIVE/NO
15. Female	70	CP	0	21	96	No	No	MR4.5	143	ALIVE/NO
16. Male	69	CP	0	-Y	3	Yes	Yes	MR4.5	20	ALIVE/NO
17. Male	60	AP	4	-Y	72	No	N/A	N/A	118	DEAD/NO
18. Male	64	CP	0	-Y	42	Yes	No	MR4.5	132	ALIVE/NO
19. Male	54	CP	1	+Y	84	No	No	MR4.5	300	ALIVE/NO
20. Male	54	CP	0	8	12	Yes	No	MR4.5	156	ALIVE/NO
21. Male	43	CP	0	8	12	Yes	Yes	MMR	20	ALIVE/NO

demonstrated by subsequent follow-up cytogenetic analyses. These results suggest that ACA in Ph- cells can be persistent.

The prognostic significance of ACAs remains unclear. Some studies have shown that chromosome 7 clonal aberrations are associated with an increased risk of evolution to myelodysplastic syndrome and acute myeloid leukemia [13]. In agreement with these reports and after a thorough follow-up, our patients with persistent monosomy 7 ultimately underwent allogeneic HCT. The significance of -Y in this setting is unclear. It has been reported that this phenomenon is a common occurrence in male individuals with aging, where up to 6% may at some time develop -Y but one of our patients was only 23 years old [14]. It is also reported that this abnormality may be significantly more frequent among patients with hematologic malignancies, suggesting that there might be an element of genetic instability [15]. In the present study, no evidence of myelodysplastic syndrome or acute leukemia was documented.

Furthermore, and in agreement with most reports, we demonstrated that the emergence of ACA's does not seem to have an effect on the outcome of CML and its treatment plan [6]. Previous studies revealed that patients with ACAs in Ph- cells did not have inferior response to TKI treatment in comparison with patients without ACAs [16]. Similarly, most cases in our present study showed satisfactory responses to TKI treatment (CCyR, MMR, $MR^{4.5}$). Sixteen out of 21 patients achieved optimal response. Two patients died, while 3 underwent allogeneic HCT due to the association of their ACAs and the possible evolution to myelodysplastic syndrome or acute leukemia (2 patients with monosomy 7 and 1 with trisomy 8). However, it should be noted that time to CCyR and MMR was significantly longer in patients that received allogeneic HCT (median 33 versus 6 months, and 48 versus 13 months, respectively). The same results were found for patients that had more than 1 ACA (median 27 versus 3 months, and 38 versus 6 months, respectively).

Conclusion

We found ACAs in 10.5% of CML patients treated with TKIs after long term follow up. Beyond previously reported ACAs, we also describe novel ACAs such as add(6)(q27), t (4;15)(q31;q26) and t(2;10)(q23;q24) that have never been reported. The significance of –Y needs to be further elucidated, since our study reports this aberration in rather young patients. Importantly, our study confirms that ACAs do not impact outcomes of CML. However, the potential of cure with allogeneic HCT in patients with monosomy 7, should be kept in mind. Larger cohorts are needed to define the significance of ACAs, since the underlying pathophysiology remains unknown.

Declaration

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Statement of Ethics: The research was conducted ethically in accordance with the World Medical Association Declaration of

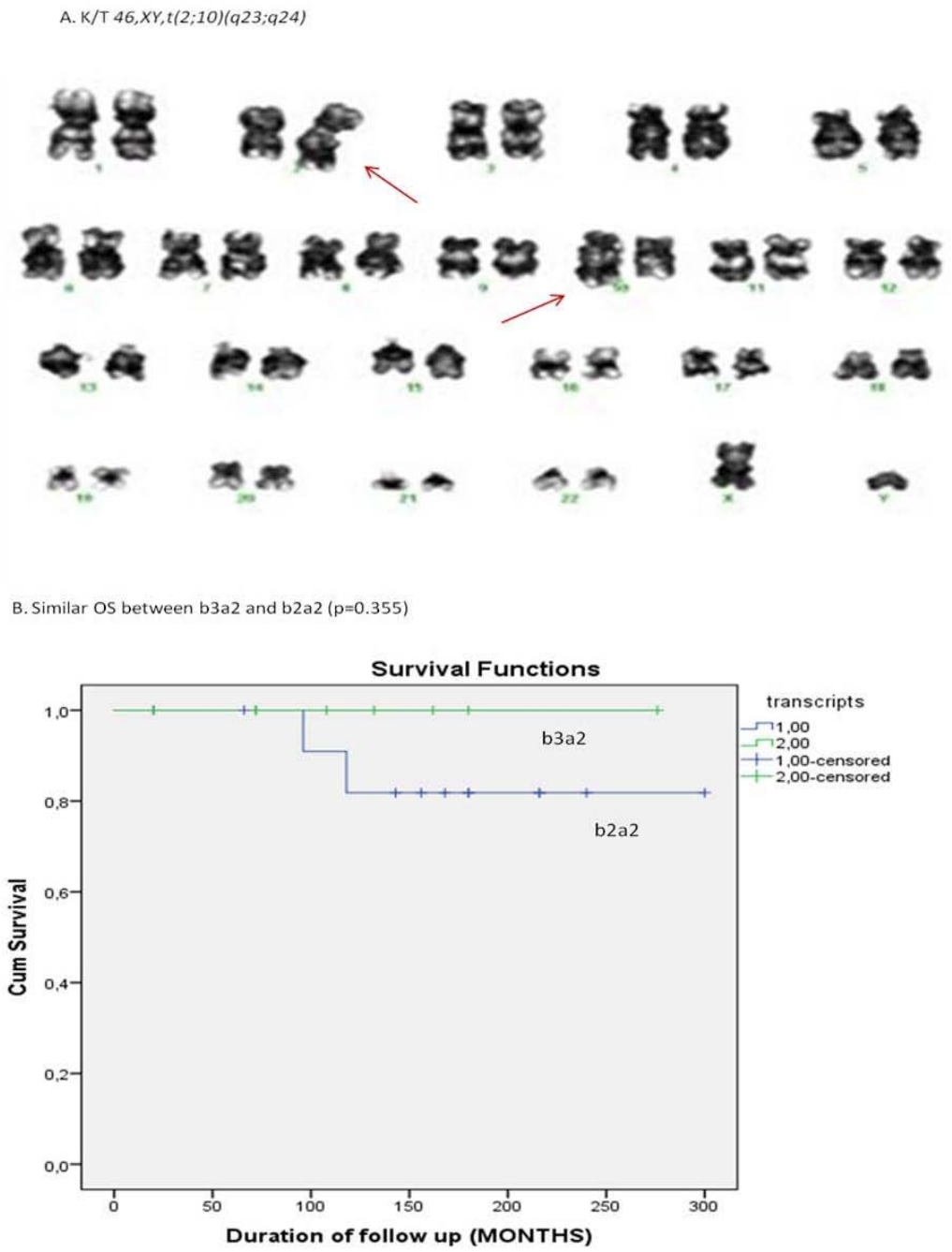

Figure 1: A) K/T 46,XY,t(2;10)(q23;q24). B) Similar OS between b3a2 and b2a2 (p=0.355).

Helsinki and in agreement with institutional guidelines. Subjects have given their written informed consent. No further ethics approval was required according to our Institution's Ethics Committee.

Authorship Contributions: C. Var, A. At and E G: Designed the study, recorded and analyzed the data and wrote the manuscript. G. P, C. Vad, C. L and N.S: Treated the patients and edited the manuscript. A. At, G. P and M. G: Performed cytogenetic analysis. T. T and A. P: Conducted PCR analysis. I. S and A. An: Analyzed the data and edited the manuscript.

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